AMENDMENTS TO THE SPECIFICATION

Please replace the Sequence Listing section filed May 8, 2002, with the Substitute Sequence Listing filed herewith.

Please replace the paragraph on page 7 between lines 6 and 28 with the following amended paragraph:

According to preferred embodiments of the aforementioned inventions, there are provided:

the aforementioned gene-mutated animal having a mutant presenilin-1 gene wherein a DNA sequence encoding around an amino acid at position 213 in an amino acid sequence of a presenilin-1 protein is mutated to the following sequence:

~~5'-TGTGGTCGGGATGATMGCC ANC CACTGGAAAGGCCC-3'~~

5'-TGTGGTCGGGATGATYGCC AVC CACTGGAAAGGCCC-3' (SEQ ID NO: 18)

wherein ~~N~~ V represents a base other than T, ~~M~~ Y represents T or C, and the underlined bases encode the amino acid at position 213;

the aforementioned gene-mutated animal having a mutant presenilin-1 gene wherein a DNA sequence encoding around an amino acid at position 213 in an amino acid sequence of a presenilin-1 protein is mutated to the following sequence:

~~5'-TGTGGTCGGGATGATMGCC ANC CACTGGAAAGGCCC-3'~~

5'-TGTGGTCGGGATGATYGCC ACC CACTGGAAAGGCCC-3' (SEQ ID NO: 19)

wherein ~~N represents C, M~~ Y represents T or C, and the underlined bases encode the amino acid at position 213; and

the aforementioned gene-mutated animal having a mutant presenilin-1 gene wherein a DNA sequence encoding around an amino acid at position 213 in an amino acid sequence of a presenilin-1 protein is mutated to the following sequence:

~~5'-TGTGGTCGGGATGATMGCC XYZ CACTGGAAAGGCCC-3'~~

5'-TGTGGTCGGGATGATYGCC NNN CACTGGAAAGGCCC-3' (SEQ ID NO: 20)

wherein ~~XYZ~~ each N independently represents A, G, T or C and NNN represents a codon as triplet bases which encodes an amino acids other than isoleucine, ~~M~~ Y represents T or C, and the underlined bases encode the amino acid at position 213.

Please replace the paragraph linking page 8 at line 25 to page 9 at line 11 with the following amended paragraph:

From further aspect, the present invention provides a plasmid comprising a DNA having a sequence of a mutant presenilin-1 gene wherein a DNA sequence encoding around an amino acid at position 213 of a presenilin-1 protein is the following sequence:

~~5'-TGTGGTCGGGATGATMGCC ANC CACTGGAAAGGCCC-3'~~

5'-TGTGGTCGGGATGATYGCC AVC CACTGGAAAGGCCC-3' (SEQ ID NO: 18)

wherein ~~N~~ V represents A, G, or C, ~~M~~ Y represents T or C, and the underlined bases encode an amino acid at position 213; and

a plasmid comprising a DNA having a sequence of a mutant presenilin-1 gene which encodes a mutant presenilin-1 protein wherein an amino acid at position 213 is substituted with an amino acid other than isoleucine in an amino acid sequence of the presenilin-1 protein and a DNA sequence encoding around the amino acid at position 213 of presenilin-1 protein is the following sequence:

~~5'-TGTGGTCGGGATGATMGCC XYZ CACTGGAAAGGCCC-3'~~

5'-TGTGGTCGGGATGATYGCC NNN CACTGGAAAGGCCC-3' (SEQ ID NO: 20)

wherein ~~M~~ Y represents T or C, ~~XYZ denotes~~ each N independently represents A, G, T, or C and NNN represents a codon as triplet bases encoding an amino acid other than isoleucine, and the underlined bases encode the amino acid at position 213. Additionally, the present invention also provides a chromosomal DNA containing exon 8 of a mutant presenilin-1 gene encoding a mutant presenilin-1 protein wherein an amino acid at position 213 is substituted with an amino acid other than isoleucine in an amino acid sequence of a presenilin-1 protein.

Please replace the paragraph on page 9 at lines 12 to 21 with the following amended paragraph:

Furthermore, the present invention provides a plasmid comprising a DNA wherein a Sau3AI site is introduced into a nucleotide sequence comprising the whole or a mutated part of a cDNA or chromosomal DNA of a mutant presenilin-1 gene encoding a mutant presenilin-1 protein in which an amino acid at position 213 is substituted with

an amino acid other than isoleucine in an amino acid sequence of presenilin-1 protein. Also provided are the aforementioned plasmid wherein the substitution of the amino acid is isoleucine at position 213 with threonine; and a plasmid comprising a DNA specified by the following nucleotide sequence:

~~5'-TGTGGTCGGGATGATMGCCACCCACTGGAAAGGCCC-3'~~

5'-TGTGGTCGGGATGATYGCCACCCACTGGAAAGGCCC-3' (SEQ ID NO: 19)

wherein ~~M~~ Y represents T or C.

Please replace the paragraph on page 10 at lines 1 to 16 with the following amended paragraph:

From further aspect, the present invention provides an embryo introduced with a plasmid comprising a DNA represented by the nucleotide sequence:

~~5'-TGTGGTCGGGATGATMGCCACCCACTGGAAAGGCCC-3'~~

5'-TGTGGTCGGGATGATYGCCACCCACTGGAAAGGCCC-3' (SEQ ID NO: 19) wherein ~~M~~ Y represents T or C; an embryo obtained by homologous recombination using each of the aforementioned plasmids; and the aforementioned embryo derived from a mammalian rodent, more preferably from a mouse. The invention also provides a primary cell culture or subcultured cell obtained by isolating a cell from the aforementioned gene-mutated animal and culturing the cell by tissue culture; a method for producing a non-human gene-mutated animal wherein the method comprises the step of transferring a mutant presenilin-1 gene by homologous recombination into an embryo of an animal, wherein the mutant

presenilin-1 gene is capable of expressing the mutant presenilin-1 and inducing production of amyloid β protein in an amount sufficient to form a progressive neural disease in a peripheral portion of the cerebral cortex of the brain; and the aforementioned production method wherein a mutant presenilin-1 protein can be expressed wherein isoleucine at position 213 is substituted with an amino acid other than isoleucine.

Please replace the paragraph starting on page 15, line 5 and ending on page 16, line 4 with the following amended paragraph:

Examples of DNAs and plasmids of the present invention include, for example:

1) a DNA comprising a mutant presenilin-1 gene encoding a mutant presenilin-1 protein wherein isoleucine at position 213 of the presenilin-1 protein is substituted with threonine, or a plasmid comprising said DNA;

2) a DNA comprising a mutant presenilin-1 gene wherein a DNA nucleotide sequence encoding amino acids around position 213 of the amino acid sequence of a mutant presenilin-1 protein is the following sequence:

~~5'-TGTGGTCGGGATGAT M GCCA N CCACTGGAAAGGCCC-3'~~

5'-TGTGGTCGGGATGAT Y GCCA V CCACTGGAAAGGCCC-3' (SEQ ID NO: 18)

wherein ~~N~~ V represents a nucleotide other than T and ~~M~~ Y represents T or C, or a plasmid comprising said DNA;

3) a DNA comprising a mutant presenilin-1 gene wherein a DNA nucleotide sequence encoding amino acids around position 213 of the amino acid sequence of an

OS-2 type mutant presenilin-1 protein is the following sequence:

~~5'-TGTGGTCGGGATGAT M GCC XYZ CACTGGAAAGGCCC-3'~~

5'-TGTGGTCGGGATGAT Y GCC NNN CACTGGAAAGGCCC-3' (SEQ ID NO: 20)

wherein ~~M~~ Y represents T or C, ~~XYZ~~ each N independently represents A, G, T, or C and NNN represents a codon as triplet bases encoding an amino acid other than isoleucine, or a plasmid comprising said DNA;

4) Any one of the DNAs or plasmids comprising said DNAs according to the aforementioned 1) to 4) wherein a Sau3AI restriction site is introduced;

5) a DNA or a plasmid comprising said DNA wherein a Sau3AI restriction site is introduced into a sequence comprising the full-length of a cDNA or a chromosomal DNA of a mutant presenilin-1 gene encoding a mutant presenilin-1 protein wherein isoleucine at position 213 is substituted with threonine in an amino acid sequence of presenilin-1 protein, or into a mutated portion of said sequence,;

6) a DNA comprising exon 8 of a mutant mouse presenilin-1 gene encoding an OS-2 type mutant presenilin-1 protein and a neomycin expression unit flanked by loxP, or a plasmid comprising said DNA; and,

7) a DNA comprising exon 8 of a mutant presenilin-1 gene encoding a mutant presenilin-1 protein wherein isoleucine at position 213 is substituted with threonine in an amino acid sequence of presenilin-1 protein and a neomycin expression unit flanked by loxP, or a plasmid comprising said DNA. However, the scope of the invention is not limited to these specific examples:

Please replace the paragraph linking page 24 at line 27 to page 25 at line 7 with the following amended paragraph:

To construct a probe for isolating a chromosomal DNA containing exon 8 of the mouse PS-1 gene, the following two oligodeoxynucleotides were synthesized:

PR-8-U: 5'-GGAATTTTGGTGTGGTCGGGATGAT-3' (SEQ ID NO: 5) (25-mer)

PR-8-L: 5'-GGTCCATTCGGGGAGGTACTTGA-3' (SEQ ID NO: 6) (23-mer)

Please replace the paragraph linking page 24 at line 27 to page 25 at line 2 with the following amended paragraph:

Example 3: Introduction of OS-2 Type Mutation

An OS-2 type mutation and a Sau3A I restriction site were newly introduced into the plasmid pX-1 using the following two oligodeoxynucleotides PRL-104 and PRL-105. Both PRL-104 and PRL-105 were 36-mers and complementary to each other:

PRL-104: 5'-TGTGGTCGGGA TGATC* GCCA C CCACTGGAAAGGCCC-3' (SEQ ID NO: 7)

PRL-105: 5'-GGGCCTTTCCAGTGG G TGGCG* ATCATCCCGACCACA-3' (SEQ ID NO: 8)

Please replace the paragraph on page 26 at line 18 to line 22 with the following amended paragraph:

Example 5: Construction of Targeting Vector Backbone

To introduce an Eag I site into the Xba I site in plasmid pmSB-0', an

oligodeoxynucleotide having the following sequence was synthesized:

5'-CTAGACGGCCGT-3' (SEQ ID NO: 21) (12 mer)

Please replace the paragraph on page 26 at lines 24 to line 30 with the following amended paragraph:

This oligodeoxynucleotide is capable of annealing via a nucleotide sequence having complementarity at the portion of CGGCCG, and forming the following sequence after introduction at a site cleaved with Xba I.

5'-TCTAGACGGCCGTCTAGA-3' (SEQ ID NO: 22)
3'-AGATCTGCCGGCAGATCT-5' (SEQ ID NO: 22)
Xba I *Eag* I *Xba* I

Please replace the paragraph on page 29 at line 1 to line 12 with the following amended paragraph:

Example 8: Isolation of ES Cells with Homologous Recombination

Colonies of ES cells that were formed in Example 7 by one-week cultivation after the addition of G418 were collected. Each colony was divided into two portions. One portion was subjected to further cultivation. For selection of clones in which homologous recombination occurred, the other portion was washed with PBS, treated with Proteinase K, and then chromosomal DNA was collected and subjected to PCR to

select clones. Nucleotide sequences of the synthetic primers used in PCR reaction were as follows.

Prsn1-2: 5'-CCCAACTCTATTTCTACCCTCGTTCATCTG-3' (SEQ ID NO: 11)

(nucleotide sequence outside the targeting vector constructed)

PKG-1: 5'-TAGTGAGACGTGCTACTTCCATTTGTCACG-3' (SEQ ID NO: 12)

(nucleotide sequence in the neo expression unit)

Please replace the paragraph on page 29 at line 13 to line 23 with the following amended paragraph:

PCR reaction was carried out for 35 cycles under the following conditions: 30 seconds at 93 °C, 1 minute at 60 °C, and 3 minutes at 68 °C per cycle. The PCR product was analyzed by 1 % agarose gel electrophoresis to identify a positive clone which gave a band at an expected position. The clone evaluated as positive was further subjected to PCR using oligodeoxynucleotides PRL-101 and PRL-102. The resulting PCR product was cleaved with Sau3A I and then subjected to electrophoresis on 2% agarose gel. Introduction of the mutation was verified by split bands, and ES cells in which desired homologous recombination occurred were selected. Nucleotide sequences of PRL-101 and PRL-102 were as follows.

PRL-101: 5'-TGCTGGAGGAAAATGTGTTATTTAAGAGCA-3' (SEQ ID NO: 13)

PRL-102: 5'-TACTGAAATCACAGCCAAGATGAGCCATGC-3' (SEQ ID NO: 14)

Please replace the paragraph on page 30 at line 9 to line 23 with the following amended paragraph:

Example 10

The knockin mouse #2 obtained in Example 9 has the heterozygous neo expression unit flanked by loxPs deriving from the targeting vector. This mouse #2 (male, about 4 months old) was mated with a F4 female of CAG-cre#13 transgenic mouse (2 months old in which transferred cre gene is heterozygous state, K. Sakai et al., Biochem. Biophys. Res. Commun. 217:318, 1997). PCR was carried out using oligodeoxynucleotides PRL-100, PRL-102 and PGK-1 under the conditions described in Example 8. A mouse from which the neo expression unit was removed was chosen as an OS-2 mutated knockin mouse without the neo expression unit (Figure 8). This mouse was heterozygous with reference to OS-2 type mutation, and had one loxP. Nucleotide sequences of PRL-100, PRL-102, and PGK-1 used for the PCR were as follows.

PRL-100: 5'-GGT CCA TCC CAG CTT CAC ACA GAC AAG TCT-3' (SEQ ID NO: 15)

PRL-102: 5'-TAC TGA AAT CAC AGC CAA GAT GAG CCA TGC-3' (SEQ ID NO: 16)

PKG-1: 5'-TAG TGA GAC GTG CTA CTT CCA TTT GTC ACG-3' (SEQ ID NO: 17)